Reference: *Biol. Bull.*, **151**: 108–125. (August, 1976)

SEASONAL GROWTH AND REPRODUCTION OF AN INTERTIDAL SPONGE, *HALICLONA PERMOLLIS* (BOWERBANK)

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Relatively little information is available on the ecological factors which influence the sequence of events leading to sexual reproduction in sponges (Fell, 1974a). The importance of temperature in regulating the seasonal breeding of marine invertebrates has long been suggested, and the subject is reviewed by Kinne (1970). Reproductive studies on sponges have related the presence of gametes, embryos, and larvae to annual temperature changes in the environment. In a field of study of *Haliclona ecbasis* from San Francisco Bay, Fell (1970) found gametes and embryos present from August to November when sea water temperatures were above 12° C. Hartman (1958), using larval settling as an index of sexual reproduction in a Long Island Sound population of *Haliclona loosanoffi*, found settling to occur when the temperature reached 20° to 22° C.

A few quantitative studies have been made on the seasonal changes in the partitioning of nutritive resources into growth and reproductive potential. Reiswig (1973) studied growth and reproduction in a Jamaican population of *Mycale* sp., and Stone (1970) followed changes in the rock surface covered by the encrusting sponge, *Hymeniacidon perleve*, simultaneously noting the proportion of the population containing embryos.

With the exception of Stone's work, the above studies were carried out on populations which were continuously submerged rather than intertidal populations which undergo fluctuating conditions. Furthermore, if one is to elucidate the mechanisms controlling reproduction in the field, precise analysis of both environmental regimes and reproductive processes is necessary. This paper presents methods for quantifying gamete production of an encrusting intertidal sponge and for estimating the true tissue temperature during the period of tidal exposure. Reproductive ouput and growth rates are then described for a population of *Haliclona permollis* located on the Central Oregon Coast. The biological observations are discussed in relation to the nutritive, salinity, light and thermal regimes of the environment.

Materials and Methods

Sampling procedure

The sponge, *Haliclona permollis*, was chosen because it is a cosmopolitan species (de Laubenfels, 1936) available all year in the easily accessible intertidal region and forms flat incrustations which are conducive to measurement. Furthermore, since it is a sessile, filter-feeding animal, its physical and nutritive environment

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can be estimated at all times. Ten to 22 pieces of sponge were collected monthly (bimonthly during spring) at stations from the rocky intertidal (+1 to +2 feet above MLLW) at Yaquina Head, Oregon over a four year period. By marking the collection site with a metal stake and recording the location of the sponges, it was possible to sample the same mass of sponge in several cases for as long as four months. However, most individuals had disappeared or grew together with their neighbor after one to two months and thus lost their identity. The collected specimens were immediately placed in Bouin's fixative. Later they were embedded in paraffin, sectioned vertically at 10 μ thickness, and stained with haematoxylin and eosin.

Quantification of gametes, embryos, and larvae

Fifteen 1.9 mm^2 microscopic sections of each sample were observed and the number of eggs, embryos, larvae, and sperm packets were counted and averaged for that month. An estimate of the number of reproductive entities per mm^3 in the above categories was obtained for each specimen by modification of a formula used by Abercrombie (1941) to compare nuclear populations of different tissues. The formula is Number per mm^3 = N $(t/D + t)$ (53), where N is the average number of gametes or embryos observed in the microscopic field, t is the thickness of the section (10 μ), and D is the diameter of the component counted. The constant, 53, is a factor converting the volume of observation (0.019 mm^3) to one mm^3. Only those eggs with a nucleus showing were counted. The average number of gametes or embryos per mm^3 in the population was calculated on the basis of both the total number of specimens of a particular sex and the total population.

Calculation of oocyte and embryo production rates

In order to achieve a dynamic description of reproduction, it is necessary to calculate the rates of conversion into the stages leading to larval production. The methods employed in studies on cell renewal systems by Olive (1971) were applied to sponges. For any time interval the change in the number of cells in the oocyte (ΔN_O), embryo (ΔN_E), and larval (ΔN_L) compartments can be described by the differences in the rates associated with each compartment: $\Delta N_O = R_O - R_E - R_{D1}$; $\Delta N_E = R_E - R_L - R_{D2}$; and $\Delta N_L = R_L - R_S - R_{D3}$. The rates are in units of number of eggs (R_O), embryos (R_E), or larvae (R_L) produced and the number of larvae released (R_S) per day per mm^3 of sponge. Data indicated that for every four oocytes produced, only one reaches the embryonic stage. Thus the rate of oocyte resorption (R_{D1}) approximates $3R_E$. Since the maximum density of embryos approximates that of the larvae, and since there is no sign of distintegrating larvae, the remaining rates of resorption (R_{D2} and R_{D3}) can be considered negligible. The rate of larval formation is much slower than that of egg formation. Thus the various rates can be computed using the difference between the monthly samples by evaluating the following formulas: $R_O = \Delta N_O + 4\Delta N_E$; $R_E = \Delta N_E + \Delta N_L$; and $R_L = \Delta N_L + R_S$. A relative value for the annual production of eggs (P_O) was calculated in units of number per cm^2 per year for an average sponge 2.5 mm in thickness by the formula $P_O = R_O$ (total reproductive days) (% females) (100) (2.5). A similar formula was used for establishing total embryos produced using R_E instead of R_O.

Estimates of somatic growth

Somatic growth was studied in terms of both the increase in biomass and the increase in substrate covered by a sponge. In the first case, the presence of active growth areas as defined using the criteria of Simpson (1968) was noted. For this mesenchymal index the specimens were scored values from 0 to 3 corresponding to a few loosely packed mesenchymal amoebocytes (0), low numbers of mesenchymal amoebocytes (1), low density mesenchyme with tracts or clumps of amoebocytes (2), and high density of mesenchymal amoebocytes (3).

Two dimensional growth by spreading over the substrate was estimated in the field by measuring the increase in the area of the flat encrusting sponges over a two week period. The outline of the sponge was traced on a piece of transparent plastic ten times and transferred to heavy paper. The shapes were cut out, weighed, and averaged to get an estimate of area. Growth was determined as the difference in areas. Since growth is related to the mass of available tissue and since the sponges studied had a fairly uniform thickness (2 to 3 mm), increases in area were divided by the total area to obtain a specific growth rate. Animals obviously broken or eaten as well as those which had grown together with neighboring individuals were eliminated from consideration.

Laboratory observations on the effects of temperature on gamete production

Sponges attached to rocks were collected in February, 1972, before any signs of gametogenesis were apparent and placed in 30 liter tanks of U.V. filtered sea water held at 4°, 7°, 9.5°, and 13° C. The tanks received approximately eight hours of fluorescent light a day. The food source for the experimental sponges was the flagellate, *Isochrysis galbana*, maintained at a concentration of about 10^5 cells/liter. Three monthly samples of the sponges were taken and processed for histological observation. On the 77th day the temperature in the 13° C tank was lowered to 10° C.

Collection of environmental data

Water and air temperatures were continuously recorded by thermoprobes installed at Whale Cove, Oregon, during the years 1970 and 1971 (Gonor and Thum, 1970). Other temperature data was obtained from the U. S. Weather Bureau at Yaquina Bay and from Yaquina Head at the time of specimen collection. Relative humidity was calculated from the difference in readings of wet and dry bulb thermometers on the days of collection. Time of exposure of the +1 foot level was obtained from water level recordings of the U. S. Weather Bureau station. Actual tissue temperatures of the sponges were measured to within 0.1° C with a hypodermic thermoprobe (Yellow Springs Instrument Co.). Total short wave insolation up to 4 μ was measured with an unshielded horizontal Eppley pyroheliometer. Salinity values for the surface sea water were obtained from Gonor, Thum, and Elvin (1970). Values for the amount of rain falling on the exposed sponges were calculated from data of the U. S. Weather Bureau.

The amount of chemically oxidizable particulates per liter of sea water was used as a measure of available nutrients. Sea water was collected up to four times each month, filtered through a 200 μ mesh screen, and collected on a 0.2 μ mesh

Teflon filter. The samples were oxidized using the dichromate method of Strickland and Parsons (1968) with glucose as a standard. To a second liter of sea water, 5 ml of Lugol's iodine solution was added, and the sample was allowed to stand a few days at 4° C. The settled diatoms were then identified and counted.

Calculation of tissue temperature

Tissue temperatures of sponges exposed by low tide were estimated by fitting the observed temperatures into an equation describing the animal at thermal equilibrium, $K_1 (T_{sp} - T_{air}) + K_2 (aT_{sp}^2 + bT_{sp} + c)(1 - Rh) = K_3 (L)$, where T_{sp} is the tissue temperature of the sponge, and $K_1 (T_{sp} - T_{air})$ is a combined term for conduction and longwave back radiation (Hutchinson, 1957). The term $K_2 (aT_{sp}^2 + bT_{sp} + c)(1 - Rh)$ is an expression for heat loss by evaporation incorporating a term for vapor pressure as a function of temperature in mm Hg multiplied by a term for relative humidity. $K_3 (L)$ is a term for heating by solar radiation. The constants K_1, K_2, and K_3 were determined by fitting measured sponge temperatures and concurrent environmental conditions into the equation. The evaporative characteristics of the sponge were determined under several conditions in the laboratory using a chamber in which temperature and humidity could be regulated. This formula for tissue temperature during exposure is an empirical one fitting the observed data, and not all the potential parameters of a heat budget were considered. Thus during exposure, the tissue temperature of a sponge for any relative humidity (Rh) is given by the quadratic solution to the heat budget equation $T_{sp} = -\frac{1}{2} Z + \sqrt{\frac{1}{4} Z^2 + ZT_{air} + 12 Z (L) - 240}$, where Z is a combination of the constants equal to $162.2/(1 - Rh)$, and L is insolation in langleys per minute. A regression of 10 pairs of predicted and observed data gave $T_{predicted} = 0.3 + 0.94 T_{observed}$, and at the 95% confidence level predicted temperatures were within 0.64° C of observed values.

A continuous temperature was then calculated for a hypothetical sponge population every hour of the day for one year. When a sponge was submerged, its temperature was that of sea water; and when exposed, the above formula was used employing a value of 80% for the relative humidity. From the continuous temperature data three thermal parameters were chosen as possibly having correlations with physiological events leading to larval production. Average daily temperature, daily maximum and minimum temperatures, and time spent above a threshold temperature were calculated for 15 day intervals.

Results

Histological samples

Examination of the histological samples enables one to determine the time of gametogenesis, the sex ratio, the rates of gamete production, and the total number of gametes produced. Single oocytes are distributed throughout the mesenchyme while sperm packets are found in clumps of three or four. Embryos are generally located near the base of the sponge often in clusters. During those periods when oocytes and embryos were most abundant the standard error of their mean densities as determined by counting 15 sections of a single specimen were on the order of

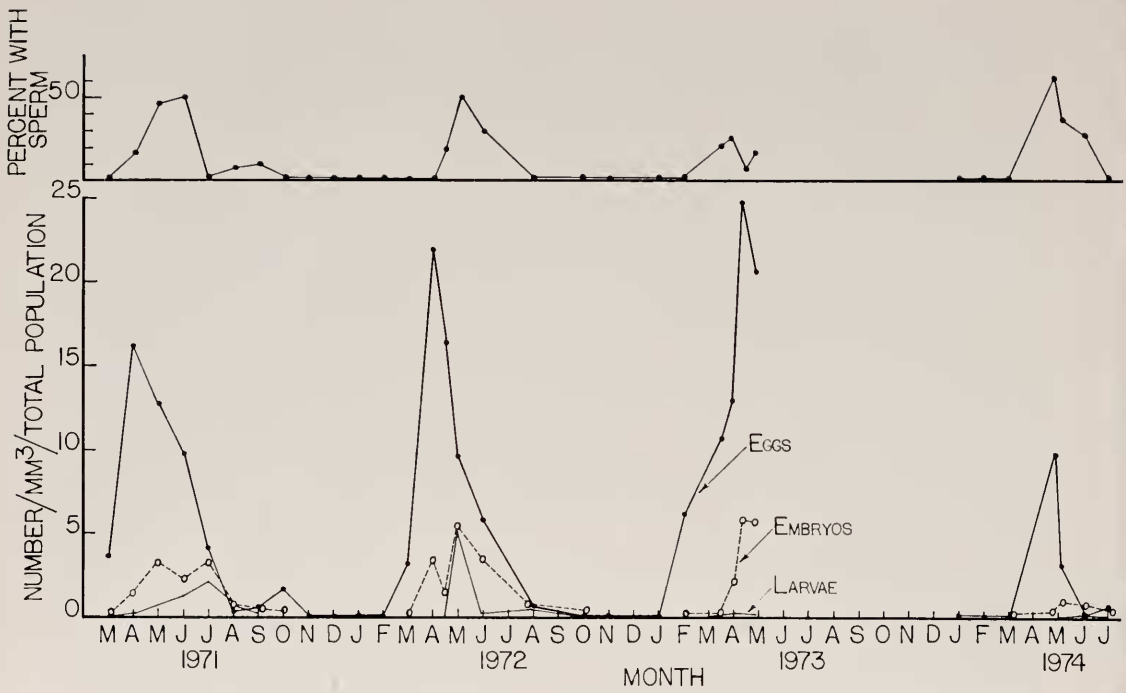

FIGURE 1. Percentage of specimens in the monthly samples containing sperm masses (upper), and the average density of eggs, embryos, and larvae observed for the total sponge population (lower). Data was not collected during the period, May to December, 1973.

5 oocytes/mm³ and 2 embryos/mm³. Within a population sample of 10 specimens the standard errors were 4 oocytes/mm³ and 2 embryos/mm³ for means near 20 oocytes/mm³ and 5 embryos/mm³. The rather high variation in embyro densities reflects a clumping tendency within the sponge.

Figure 1 shows the monthly occurrence of eggs, embryos, and larvae in the total sponge population and the percentage of specimens with sperm masses. Oocytes were first seen in the March samples during 1971 and 1972. However, in 1973 they were present as early as February 13, and in 1974 they did not appear until April 26. The maximum oocyte density for 1974 is also lower than the previous three years;

TABLE I

Sex ratio in late spring populations of Haliclona permollis *at Yaquina Head, Oregon.*

Year	Collection dates	N	Males	Females	Indifferent	Chi²	Significance*
1971	May–June	25	12	13	0	0	No
1972	May–June	13	5	7	1	0.16	No
1973	April	24	3	20	1	9.6	Yes
1974	April–May	32	15	9	8	1.04	No

* Significant difference from a ratio of 1:1 at the 5% level.

TABLE II

Rates of oocyte and embryo production per female Haliclona permollis *at Yaquina Head, Oregon.*

Year	Period	Rates (number/mm³/day)	
		Oocytes	Embryo
1971	Mar–April	+1.30	+0.07
	April–May	+0.49	+0.14
1972	Feb–Mar	+0.30	0
	Mar–April	+1.90	+0.23
	April–April	+0.77	−0.03
	April–May	+0.11	+0.52
1973	Jan–Feb	+0.32	0
	Feb–Mar	+0.31	0
	Mar–Mar	+0.53	+0.39
	Mar–April	+1.43	+0.28
	April–April	−0.24	0
1974	Mar–April	+0.71	+0.01
	April–May	−2.00	+0.22

however, this decrease reflects a change in the sex ratio rather than a change in the number of oocytes produced per female. During the first three years the earliest embryos were seen in April approximately one month after the appearance of oocytes. Larvae are first observed in May, but their pattern of release differs over the years. Some sperm masses were usually present by the time embryos were produced; however, the maximum sperm density was generally found in May, one month following maximum embryo density. In 1972 a high embryo density was found in the April sample which did not contain males.

The number of males and females in the monthly samples at a time when the sex was known for a majority of the specimens is presented in Table I. In 1971 and 1972 the sex ratio was 1:1, but in 1973 there were significantly more females than males. In contrast there were either fewer females or an abnormally large number of sponges which did not produce any gametes in 1974. Over the entire four years only one (0.7%) simultaneous hermaphrodite was found among the 147 specimens exhibiting gametes out of a total of 342 specimens. In 1974, 17 specimens were collected in which the microhabitat could be definitely classified as totally shaded or totally exposed to the sun during low tide. While 37% of the males were on rocks exposed to the sun during low tide, only 11% of the females were found in such a situation.

Calculated rates of egg and embryo production are given in Table II. The maximal rates appear to be between 1 and 2 eggs/mm³/day/female and 0.2 to 0.5 embryos/mm³/day/female. Since the variabilities of the counts upon which the rates are based are high, the order of magnitude is of greater significance than the actual values. In Table III, we see that while the total number of eggs in females is nearly a constant 44/mm³, over the years the number of embryos varies eightfold. The difference in relative annual production of embryos for the whole population rises to fifteen times when fluctuations in the sex ratio are considered.

TABLE III

Annual production of oocytes and embryos for the Haliclona permollis *at Yaquina Head, Oregon.*

Year	Female production (number/mm³/year)		Percentage of females	Relative annual production in total population (number/cm²/year)	
	Oocytes	Embryos		Oocytes	Embryos
1971	44	5.7	52	5730	745
1972	46	19.6	54	6190	2640
1973	47	8.4	83	9750	1780
1974	38	2.4	28	280	177

Constant temperature experiments

Table IV presents the results of the laboratory observations on the reproductive state of specimens after 77 days at four constant temperatures. Clearly 4° C for any extended period is a lethal condition. The number of specimens which did produce gametes was too small to show significant differences between the various temperatures but some information can be gained from the results. It is clear that sperm can be formed at any temperature between 7° and 13° C, although sperm packet density was much greater at 13° C. Spermatogenesis always began after oogenesis. Oocytes were produced at the rates of 0.04 and 0.16 oocytes/mm³/day for temperatures of 7° and 9.5° C, respectively, but these rates are only a tenth of those in the field. Embryos were never produced although males and females were in the same tanks. Most interesting was the appearance of abnormal oocytes in the sample in which the temperature was reduced from 13° to 9.5° C. These oocytes differed from normal eggs by having, in addition to the nucleolus, a large eosinic granule in the nucleus. Sponges which did not produce gametes were found to be in a state of regression.

Observations on growth

The observations on the somatic growth indicators are presented in Figure 2. Mesenchymal densities with an index above 1.2 were considered to be significantly

TABLE IV

Histological characteristics of sponges after 77 days submergence at constant temperatures in the laboratory.

(February 14 to May 2, 1972)

Temperature (° C)	N	Mesenchymal index	Number of oocytes	Date of first appearance	Number of sperm	Date of first appearance	Number without gametes
4	4	1.4*	0	—	0	—	4
7	6	1.0	2	Mar 25	2	May 2	2
9.5	8	1.7	2	Mar 2	2	May 2	4
13	6	0.7	0**	—	4	May 2	2**

* Died within 30 days.

** Abnormal oocytes and two hermaphrodites produced *after* cooling to 10° C.

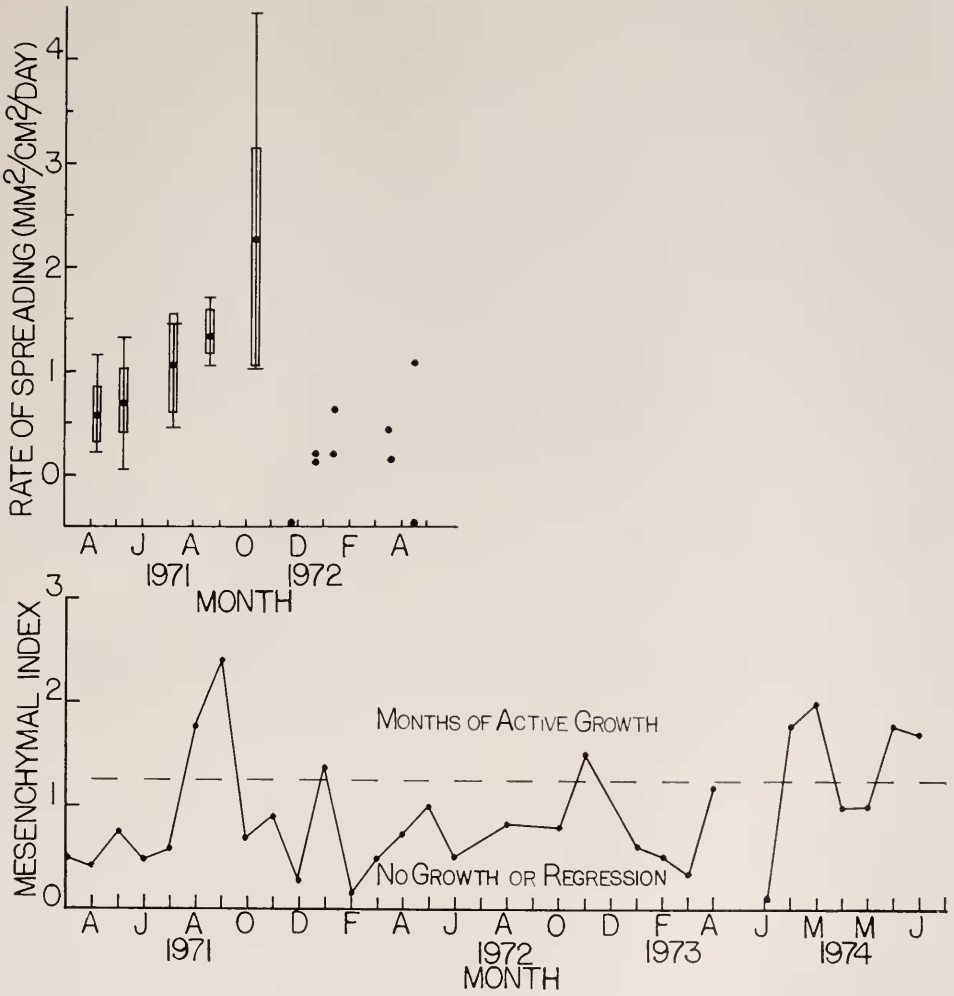

FIGURE 2. Rate of spreading or increase in area covered by a sponge (upper) showing average, 90% confidence intervals, and ranges. Dots indicate values for single individuals. The average mesenchymal index (lower) for monthly samples is shown in which specimens with loosely packed cells were given a value of 0, with low density amoebocytes a value of 1, with clumps and tracts of amoebocytes a value of 2, and high density amoebocytes a value of 3. Values above the dashed line (index = 1.2) demonstrate a significant increase in mesenchymal density.

different from nongrowth values. In 1971, increase in the mesenchymal amoebocytes in August and September preceded spreading by one month. During late fall of 1972 there was again an increase in mesenchymal density. 1974 appears to be an abnormal year since an increase in amoebocytes occurred during February. An average spreading rate during summer and fall is on the order of 1 mm^2/cm^2/day. During November spreading suddenly ceased and in some cases regression was measured. From October to December the rate of specimen disappearance from the

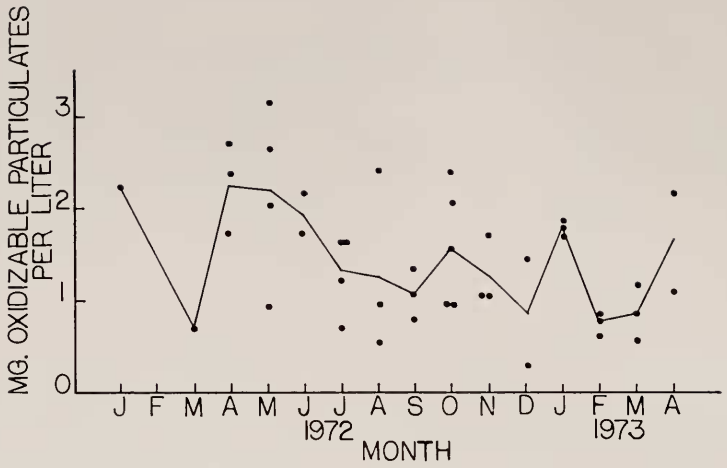

FIGURE 3. Chemically oxidizable particulate material (0.2 to 200 μ) suspended in the near-shore water in units of mg glucose per liter. Monthly averages are connected by a line and the values of samples collected one week apart are represented by dots.

marked field specimens was about 1.5% per day, while in April and May it dropped to 0.86% per day. Production of gemmules was not observed in histological sections although gemmules from this species were occasionally present in the field.

Environmental factors

Growth and reproductive trends were studied in relation to the annual salinity, nutrient, light, and tissue temperature regimes. Extensive rain, especially during tidal exposure, results in hypo-osmotic conditions. The potential rainfall on exposed sponges was calculated by multiplying the total inches per day by the fraction of the day during which exposure takes place. The heaviest rainfall occurs from November through February. During January and February surface seawater salinity may drop as low as 20‰ on particular days, but the rest of the year the values stay between 30 and 34‰. The crucial period of February to April had significantly lower rainfall (13 inches) in 1973 compared to 1972 (26.7 inches) and 1974 (29.1 inches). Long periods of exposure in the sun during May to July when the low tides occur during the middle of the day lead to dessication and consequently hyper-osmotic conditions.

Nutrients available to the sponge population as measured by the amount of oxidizable particulate material ranging in size from 0.2 to 200 μ had values between 0.2 and 3.2 mg glucose equivalents per liter (Fig. 3). High values in the spring are associated with diatom blooms increasing from 10^3 cells per liter to 10^4 and 10^5 cells per liter beginning April 1, 1972 and April 11, 1973, respectively. Occasionally in the fall and winter terrestrial runoff and wave mixing of bottom detritus result in high amounts of both oxidizable and inorganic particulates. It is clear from Figure 3 that monthly averages have little significance in light of the possible threefold difference between two successive weeks.

The characteristics of sunlight falling on the +1 foot level during tidal exposure are a function of both the tidal regime and incident solar insolation. For this collecting site the value of impinging sunlight rises rapidly in March and reaches a maximum in May.

Intertidal sponges have three possible thermal regimes. The submerged sponge has a tissue temperature always equal to that of sea water. The sponge subjected during tidal exposure to direct sunlight is consequently heated, and the sponge in the shade during tidal exposure undergoes slow heating or cooling depending on air temperature, wind speed, and humidity. An example of a rapid change in tissue temperature is shown in Figure 4 in which a sponge was initially exposed in the shade for several hours and then exposed to the sun shortly before it was covered by the incoming tide. In this case, heating was a rapid 13° C per hour. A nearby specimen in the shade remained at the wet bulb temperature.

Continuous tissue temperatures of hypothetical sponges were calculated for the year 1970 to 1971. For the continuously submerged population, 24 hourly temperatures each day were combined to give an average daily temperature, and these daily temperatures were averaged for 15 day intervals. The result is an average temperature remaining within two degrees of 10° C (Fig. 5). Sponges exposed in the shade or sun have average values within 1° C of the submerged values. These deviations from the submerged average are small due to the relatively short 0 to 6 hour periods of exposure each day at the +1 foot level, and there

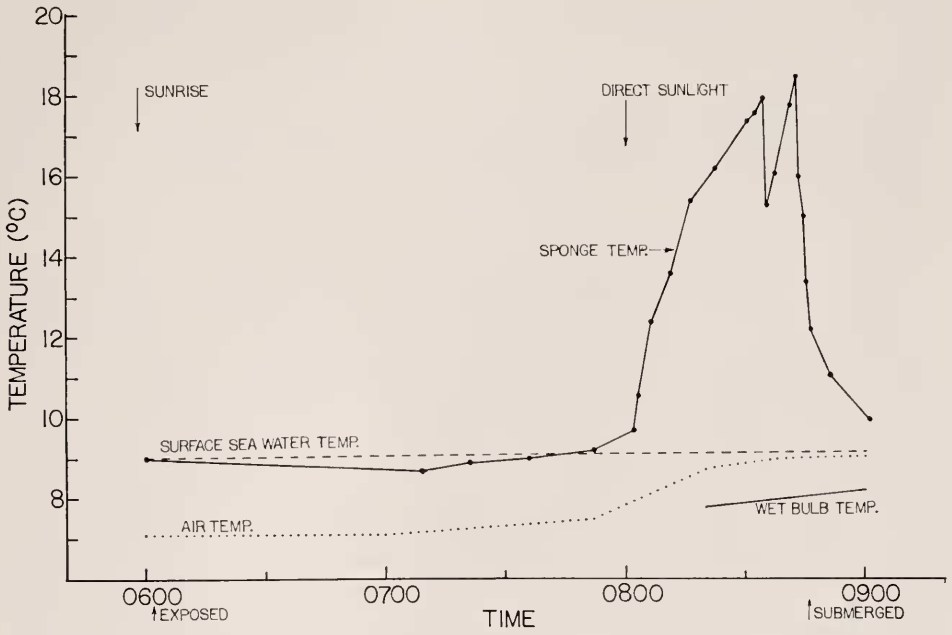

FIGURE 4. Tissue temperature of a sponge which has been exposed by the outgoing tide. Initial exposure was in the shade and direct exposure to sunlight occurred at 0800 hours. The sudden decrease at 0830 hours was caused by a large wave. A nearby sponge in the shade remained at the wet bulb temperature.

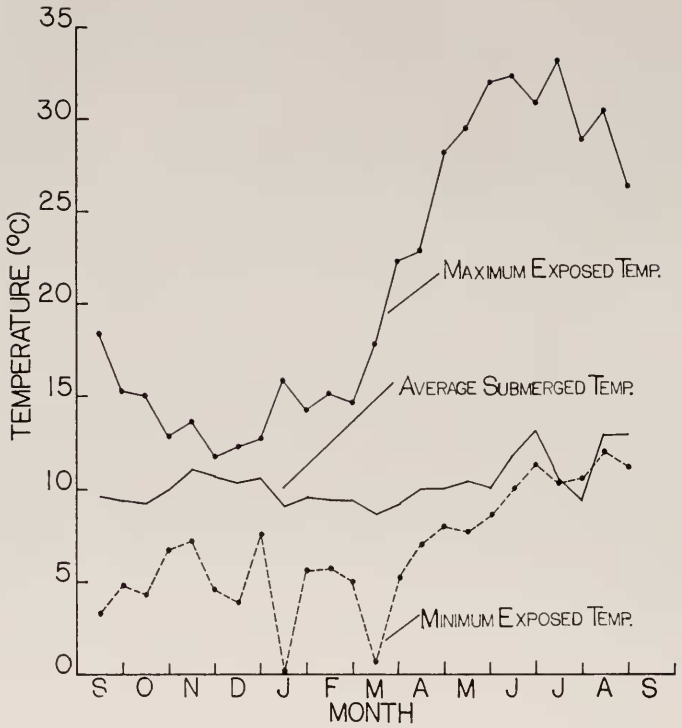

FIGURE 5. Annual tissue temperatures of sponges showing the average temperature of submerged populations and the maximum and minimum temperatures reached each 15 day period by exposed populations. Average tissue temperatures of the exposed populations were found to be within a degree of the submerged group and are not shown. This data covers the period 1970 to 1971.

is no significant difference between the average temperatures of the three regimes.

As has been shown, the temperatures reached during the period of exposure can be far from those of sea water. The maximum and minimum tissue temperatures attained in each bimonthly period are also recorded in Figure 5. Heating during tidal exposure occurs in late March, and temperature differences of 10° C begin to occur at this time. The minimum temperatures approaching 0° C in January and March should not be neglected in an analysis of the thermal regime. A better indication of the thermal regime can be obtained by calculating the amount of time spent above arbitrary threshold temperatures. Threshold values of 10° and 12° C demonstrated significant annual changes in late March and in late May, respectively, for those sponges exposed directly to sunlight. Although maximum exposed temperatures of 32° C were predicted for sponges exposed during the summer, very little time was spent above 14° C. The great variation during late summer and fall reflects intermittent periods of cooler water due to coastal upwelling. Average values for sea water and air temperatures during the years 1971 to 1974 were compared, and it was found that both air and sea water temperatures during February of 1973 were 1.5° to 2.0° C warmer than those of other years.

Relationship between environmental events and reproductive behavior

The relationship between environment and reproductive events must be presented for later discussion. Oogenesis in the *H. permollis* population was first observed in late February to early March. During this period in 1971, freshwater stress from rain declined and tissue temperature was at its lowest. Particulate nutrient concentration is also at a minimum. In fact, in 1972 and 1973, eggs were formed well before the diatom bloom, and no direct relationship exists. Examination of the continuous tissue temperature calculation (Fig. 5) and consideration of the monthly values of sunlight impinging on the +1 foot level for the year 1970–1971 shows that initiation of oogenesis occurs with increase in sunlight during the February to March period rather than temperature. For the four year period, 1971 to 1974, however, comparison of the average February air and seawater temperatures with the reproductive data supports a correlation of either tissue temperature or light with the onset of oogenesis. 1973 was a year of early oogenesis and had higher temperatures, less rain, and probably increased incident radiation. During 1974, a year of late oogenesis, there was much rain and consequently reduced insolation and low temperatures.

Although the maximum rate of oocyte formation occurs in late March to early April when there is an increase in the time the sponge spends above 10° C, there is no correlation between rate of oogenesis and average tissue temperature over the years.

Spermatogenesis is better related to thermal changes than oogenesis. Sperm masses are first seen in April when both impinging light and the length of time spent above the 10° C threshold by sponges exposed to the sun increases further. Sperm were observed earlier (March 17) in the warmer year of 1973. In the laboratory, sperm were first observed on May 2, slightly later than in the field specimens. The density of sperm masses produced was greater at 13° C than at the lower temperatures, although it should be recalled a few sperm packets were produced at 7° C. There is no apparent relationship between the initiation of spermatogenesis or sperm release and the concentration of oxidizable particulates. Rapid disappearance of sperm in males occurs from late May to early July when maximum exposed temperatures exist but the sea water temperatures may be quite cool due to upwelling.

There is a positive relationship between embryo production and the amount of oxidizable particulates during the years 1971 and 1972. Embryo production, unlike oocyte development, involves accumulation of materials, and available nutrients would be expected to influence the process. Comparison of Table II and Figure 3 shows that the rates of embryo production, 0.52 and 0.28 embryos/mm^3/day, for the years 1972 and 1973, can be correlated with the average amount of oxidizable particulates in the water, 2.2 and 1.5 mg glucose/liter, respectively. The total amounts of embryos produced during these years, 19.6 and 8.4 embryos/mm^3/female, respectively, also show this relationship.

Discussion

Analysis of growth and reproduction of the intertidal sponge, *Haliclona permollis*, over a four year period reveals variation in the onset of gametogenesis and

the quantities of gametes and embryos produced. The temporal and quantitative aspects of reproduction and growth can be related to changes in the impinging sunlight, nutrition, and tissue temperature of the organism. These changes in microhabitat are in turn influenced by several primary environmental factors. Both quality and quantity of particulate food are affected by terrestrial runoff, wave mixing, and spawning or death of other organisms as well as the response of primary producers to sunlight. The tissue temperature of the sponge is determined during tidal exposure by sunlight, tidal regime, relative humidity, wind speed, and the air temperature, whereas during submergence it is determined only by seawater temperatures. The existence of subpopulations with respect to the environment further complicates any interpretation of reproductive behavior.

An explanation of sexual reproduction in sponges must describe those intrinsic and environmental factors which initiate gametogenesis, control sexual differentiation, and influence the rates of gamete production. In sponges with a dominant gemmule stage, there may exist a regulatory connection between reproduction and gemmule germination (Fell, 1974b). Gilbert (1974) found oocyte formation occurred throughout the year within a week after placement of the gemmules of the freshwater sponge, *Spongilla lacustris*, into a lake and concluded that egg production was under endogenous control not requiring environmental stimuli. Fell (1974a) found for the marine species, *Haliclona loosanoffi*, that sexual reproduction was initiated very soon after gemmule germination, but he points out that this species is the only marine sponge known in which gemmules persist exclusively for part of the cycle. In contrast, on the Oregon coast, specimens of *H. permollis* with adult tissues are always present during midwinter although gemmules are occasionally found. If spring reproductive behavior were a consequence of gemmulation the previous fall, the larger number of specimens lacking sexual characteristics in 1974 might be ascribed to low gemmule production the previous year. However, such a system would not explain the reproductive success of earlier years when there was no particular increase noted in gemmules. Initiation of gametogenesis in *H. permollis* appears environmentally controlled, but the stimulus could still involve a short period of somatic growth in those sponges surviving the previous winter.

Initiation of gametogenesis is really the onset of differentiation of archaeocytes or choanocytes into primary oocytes and spermatocytes (Fell, 1974a). This differentiation and the beginning of meiosis in *H. permollis* is not a continuous process but occurs once a year for a brief period of two weeks or less in duration. Incident light during a crucial late February to early March period is the environmental parameter most closely related to the initiation of oogenesis. The correlation with increasing temperature is poor since in 1971 the period of oogenesis occurs during March, a time with the lowest average tissue temperature (9° C), but the possibility of a cold stimulus cannot be overlooked. While the less precise environmental data of 1972, 1973, and 1974 support a thermal effect, they also support the hypothesis of a light cue. Assuming that rain is an indication of cloud cover, increasing amounts of light in 1974, 1972, and 1973 correspond to earlier appearance of gametes on April 26, March 14, and February 13, respectively. The quantity of light falling on the shaded and nonshaded populations during tidal exposure will of course be different, but the trends for both populations will be the same. The

increase in light level for the sponges to a maximum in May results from tidal exposure shifting to the middle of the day coupled with the insolation increasing as the year progresses. The suggestion in this paper that light directly affects sponge physiology is supported by the observations of Rasmont (1970), who found a positive effect of light on respiration and an inhibitory effect of light on the gemmulation of a freshwater sponge. Since the sponge did not possess symbiotic algae, Rasmont suggests that sponge cells themselves are photosensitive.

Many sponges can potentially express the characteristics of either sex. *Haliclona* and the related genus *Reniera* contain examples of both successive and simultaneous hermaphrodites (Fell, 1974a). The basis of this hermaphrodism could be at the genetic level or due to the coalescence of larvae as reported for *Ophlitaspongia seriata* by Fry (1970). However, only 0.7% of the *H. permollis* specimens were found to exhibit both embryos and sperm compared to the 6% found by Fell (1970) for *H. ecbasis*. The question arises as to how one sex becomes favored over the other. A previous study on *Microciona prolifera* has shown higher thermal thresholds for sperm formation than for oogenesis (Simpson, 1968). Although several independent pieces of data in this report suggest that warmer temperatures favor sperm formation in *H. permollis* and are above the optimum for oogenesis, none of them are statistically conclusive. More males and higher sperm densities occurred at 13° C than at 7° and 9.5° C, males are often seen later in the year than females when tissue temperatures are higher, and only 11% of the females in 1974 were found in positions exposed to the sun while 37% of the males were found in this microhabitat. However, there is conflicting evidence for the thermal initiation of gametogenesis. Both types of gametes were in fact formed at 7° C, and abnormal eggs were formed in males following a temperature drop. Furthermore, a comparison of the sex ratios between the years contradicts the hypothesis of thermal sex determination since more females were present during the warmer 1973 and significantly more males in the cooler 1974. Finally, the field data in this study suggest that neither rates of oogenesis nor the total amounts of eggs produced are influenced by temperature.

If sponges are hermaphroditic, and if sexual differentiation is truly thermally controlled, then a dilemma arises since in the field sponges destined to become males must pass through a period of low temperatures favorable to oogenesis before reaching that optimal temperature for sperm production. The dilemma could be solved if the population is asynchronous with respect to the initiation of gametogenesis either physiologically or due to microhabitat differences. Those sponges that begin gametogenesis early in the year form oocytes and those that begin later form sperm. Gametes once formed could inhibit further gametogenesis as was suggested for the freshwater sponges (Gilbert, 1974).

Such a system may be related to the greater bioenergetic costs of oocyte and embryo production relative to sperm production. As Figures 1 and 2 show, somatic growth and reproduction appear to be mutually exclusive. Although the rate of growth was not separated by sex, 1973, a year with many females, showed a low mesenchymal index, and 1974, a year with many males, showed a higher mesenchymal index. Clearly a larger biomass is beneficial for egg production. Those individuals which have increased in size before the reproductive period may be better suited for egg production and are induced to do so by either increasing light or temperature.

Any individual which has survived the winter and is in at least its second year would therefore have a greater chance of producing eggs. The sponges from the larvae of the previous year would be smaller and grow in the spring before producing sperm. The large number of females in 1973 could have given rise to a large surviving larval population which then became the increased number of males the following year. Such an explanation would incorporate the features of the low density and size advantage models for hermaphroditism as discussed by Ghiselin (1969). However, the situation is not that simple since as in 1974 it is possible for a large percentage of the population to have no sexual expression. In order to fully understand the sexual sequence of this animal the same individual must be sampled for at least two years. Although this study followed specimens from the same rocks for four years, it was not possible to keep track of individuals. It is clear that average tissue temperatures of these Oregon sponge populations do not have the large annual differences reported in similar studies and as a result temperature changes may not be the best cue for reproduction. In summary, the thesis that temperature is the primary controlling factor for sexual reproduction in marine sponges should be reexamined.

The appearance of embryos obviously requires the earlier presence of oocytes, but it is unclear whether embryogenesis always requires fertilization. Gilbert (1974) states that fertilization is necessary for cleavage in *Spongilla lacustris*. Neither fertilization nor cleavage stages were observed in *H. permollis* although multiple nuclei were occasionally seen in a few embryos. As Fell (1969) has shown for *H. ecbasis*, the oocytes engulf numerous nurse cells which obscure most of the detail of the oocyte activity and presumed fertilization. In 1971, 1973, and 1974 embryos were found at the time of the first appearance of sperm. The apparent presence of embryos before the appearance of sperm in 1972 may be due to the fact that these early structures are really oocytes which have engulfed a large number of nurse cells. If decrease in sperm density is any indication of the time of sperm release, then fertilization occurs from May to June.

No correlation was found between the rate of embryo production and the average air and seawater temperatures, but a positive relationship between rate of embryogenesis and the amount of oxidizable particulates in the water does exist. Embryo growth is determined by nutrients through the engulfment of nurse cells and possibly by the rate of synthesis of certain storage products. Reiswig (1972) analyzed incurrent and excurrent water from Jamaican sponges and suggests that the unresolvable particulate fraction of which colloidal materials form a major part are an important nutritive source for sponges. Therefore, the concentrations of the larger particles followed in this study may not have direct relationship to sponge nutritive uptake. During periods of high primary production or spawning by other animals, a large amount of colloidal material is also produced. The failure to produce embryos in the female laboratory specimens could have resulted either from lack of sperm release or the flagellates being an inadequate diet.

An approximation of the material allocated to reproduction can be made based on volumes. Reiswig (1973) calculated for the Jamaican sponge, *Mycale* sp., that 2.3% of the volume was involved in production of the large 2.6 mm embryos. For *H. permollis*, the smaller 0.18 mm embryos compose 1.5% of the total volume, and up to 6.3% is found in sperm masses in contrast to the 5% to 10% found by

Reiswig. The similarity between the values is remarkable in light of the different species and environments of the two populations and may reflect a physiological characteristic of the material and energy budgets of sponges. It should be noted that the amount of cellular material present in a cubic millimeter of sponge is variable. The somatic biomass certainly influences the number of embryos produced since it is related to the amount of collected and accumulated nutrients, and it should be a consideration during further quantitative study of sponge reproduction.

As would be expected, somatic growth also shows variation throughout the year. During the fall of 1971, an increase in somatic growth detected as spreading of the sponge on the rock surface occurred. Minimal growth and often regression occurred from December until April. Although maximum spreading rate was found in the fall, growth and reproductive processes overlap since some spreading did occur during the spring. The rates presented (1 mm^2/cm^2/day) or 1% per day are similar to the 0.3 to 0.5% found by Stone (1970) for another encrusting sponge when his area index data is converted to similar units. For the more massive *Mycale* sp. in Jamaican waters, Reiswig found an annual growth rate of 60% for mature specimens. In contrast, *H. permollis* could have three times this rate if it was allowed 100 growing days a year. Such a difference probably reflects the requirement of the temperate intertidal sponges for a fast growth rate to compensate for physical destruction during harsh winters and for grazing of limpets and nudibranches (Elvin, 1976). It will be recalled that the death rate during the winter was estimated at 1.5% per day.

During 1971 and 1972 some increase in cell density was in evidence in the spring, but growth mainly occurred in the late fall (October to November). Increases in mesenchymal index for February, 1974, a year in which oogenesis was particularly low, are interesting since rainfall that month was about four times that of the high reproductive, low growth period of 1973. In addition to hypo-osmotic stress, this stormy period also would have had high concentrations of inorganic particulates which may cause clogging of the sponge. Thus instead of a period of increased growth, it is possible that February, 1974 was a period of stress and that the rise in mesenchymal density was a result of regression rather than proliferation. During early March new individuals are generally found even though reproduction has not occurred. It cannot be stated whether these sponges result from outgrowths of remaining adult tissues in cracks of the rocks, colonies arising from larvae of the previous fall, or germination of gemmules.

Dissection of the intertidal environment of *H. permollis* over a four year period has revealed a complex set of parameters which can be related to growth, timing of reproduction, and the quantity and quality of gametes. A more precise description of the intrinsic and environmental factors influencing reproduction would require large bimonthly samples in order to detect the degree of asynchrony in the population as well as differences in the behavior of various subpopulations. The variabilities within the samples of this study indicate that in order to obtain a value for embryo density within 20% of the true mean with 90% confidence about 70 microscopic sections or 1.33 mm^3 of each specimen would be required. Furthermore, a field sampling of 30 to 40 specimens would be required for the same statistical criteria. Data on the direct effect of incident light must await the development of techniques for long term laboratory culture of marine sponges.

Appreciation is expressed to Patricia J. Elvin for assistance with the histological preparations and in preparing this manuscript. Dr. Jefferson J. Gonor, Marine Science Center, Newport, Oregon, generously donated laboratory space and materials for this study. This work is the result of research sponsored in part by the Oregon State University Sea Grant College Program, supported by NOAA Office of Sea Grant, Department of Commerce, under Grant #04-3-158-4.

Summary

1. Somatic growth and reproductive characteristics of an intertidal sponge, *Haliclona permollis*, were followed over a period of four years in a population on the central Oregon Coast.

2. Methods have been developed for estimating the instantaneous tissue temperature of sponges, calculating egg and embryo production, and measuring somatic growth rate.

3. Initiation of oogenesis during early March is best related to increases in incident light.

4. A maximum rate of oogenesis (1.5 eggs/mm^3/day) is found near the first two weeks of March, and the annual oocyte production was constant at about 44 oocytes/mm^3.

5. Temperature appears to have a secondary role in reproductive behavior but may influence sexual expression.

6. Development of embryos is related to particulate food supply in late spring.

7. Somatic growth rates are minimal from December to April and reach a maximum average of 1% per day in the fall.

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